A HISTOLOGICAL STUDY OF THE DIGESTIVE SYSTEM OF ASCAPHUS TRUEI (STEJNEGER)

by

JOHN CLAIR BELTON

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INTRODUCTION

Ascaphus represents a unique form showing many characteristics of both the Salientia and the Urodeles. Taxonomically, the genus *Ascaphus* is included in the order Salientia of the class Amphibia. The most primitive representatives of this order living today are included in the suborder Amphicoela, so named because the interdorsals and interventrals remain cartilaginous as in the majority of Urodeles (13, p. 485). One is likely to get an entirely wrong impression of the vertebrae of *Ascaphus* if he accepts Noble's description of the amphicoelous vertebrae. According to Ritland, the use of the term amphicoelous for the Ascaphidae is confusing and misleading since the vertebrae do not naturally assume the biconcave shape, but rather when successive vertebrae are broken apart, the intervertebral cartilage is ruptured leaving a surface which is nearly flat (16, p. 131). In *Ascaphus*, the perichondral cartilage develops into cartilagenous centra that may be considered as juvenile since this flexible cushion element ossifies in most other Salientia (16, p. 130).
The taxonomic relationship of *Ascaphus* has stimulated numerous comparative anatomical studies. The literature has concentrated interest on the gross anatomy or on specialized structures of these animals. A comprehensive and valuable work by Noble includes observations on the homology of limb girdles in different species (10). Pusey (15) discusses the development and structure of the chondrocranium and jaws. The cranial anatomy of *Ascaphus* was later studied by De Villiers (6). This work put more emphasis upon the histology and development of buccal and nasal glands. In 1955 Ritland published a very comprehensive and detailed account of the post-crani al skeleton, spinal nerves, and myology (16 and 17). The complete structure and development of the pelvic region was reported in two papers by Van Dijk (21 and 22).

The similarities uniting the Urodeles and *Ascaphus* may represent a retention of an ancestral amphibian plan which led to the present Salientia and Urodeles. Pusey lists twenty-six characteristics of head structure that *Ascaphus* shares with the Urodeles. Pusey reported, (15, p. 179) "Among the frogs *Ascaphus* is shown to be most nearly related to the Discoglossidae, which appear to have been derived from an ancestor with many
Ascaphus-like characters." This statement is in agreement with Noble's classification and is substantiated by the sixteen anatomical similarities in head structure and development shared by the discoglossid frogs and Ascaphus (15, p. 175).

Caution must be taken, however, not to classify any organism as "primitive" or "specialized" from observations made on a limited portion of the body or its development. The word "primitive" may justifiably be used in reference to characteristics that unite the animal to its ancestral form. It was this view that led Ritland to say, "In a strict sense to consider any living animal primitive is a little naive and reflects a common misconception" (17, p. 208).

Although frequently designated as the most primitive Salientia, Ascaphus in its unique but uniform habitat has become highly specialized. The remarkable larval and adult adaptations for life in mountain torrents have been well summarized by Noble (11). The larvae are characterized by a flattened, tapering body and a long, powerful tail with a narrow fin, a large sucker used by the larvae as a hold-fast, and the absence of lungs. With reduced auditory apparatus, simple sac-like lungs and external genitalia in the male which facilitate fertilization, the adults are just as specialized.
According to Francis (7, p. 362) the alimentary tract and associated structures of frogs show a general anatomical similarity to those of salamanders. However, there is in Salientia (1) an absence of teeth on both jaws and a reduced number of pre-vomerine teeth, (2) a mobile tongue, (3) eustachian tubes, (4) a liver more complex and sub-divided into more lobes than that found in salamanders. Bowers (3, p. 265-274), who studied histogenesis in the intestinal tract of *Bufo*, described the gradual shortening and narrowing of the intestinal tract characteristic of frog embryology. He concluded that these larval changes are produced in conjunction with the changes from herbivorous to carnivorous diet.

Numerous studies have been made upon the morphology of *Ascaphus* with special emphasis on the skeletal system, the cloaca, and the muscles. The literature includes many anatomical comparisons of frogs and urodels but further description of the internal anatomy is needed; only portions of the digestive system have been studied and these are discussed as incidentally associated with other structures. The present study is an endeavor to describe and analyze aspects of the digestive tract and its associated glands on a macroscopic and microscopic level. An understanding of the internal anatomy and
morphogenesis will supplement the literature and make taxonomic comparisons more meaningful.
METHODS AND MATERIALS

Fifty-seven adult specimens of *Ascaphus truei* were collected from the south fork of the MacKenzie River above Dutch Oven Forest Camp. The frogs were promptly transported to the laboratory and held in one-gallon storage jars at a temperature of 16° C. until used.

Ten adult specimens, five males and five females, were studied by gross dissection. Measurements, including length and diameter, were made on each portion of the digestive tract. The wet weight of the liver was recorded for each frog, but the small amount of pancreatic tissue made such recording unfeasible.

The small size of the frogs made fixation of tissues by perfusion through the circulatory system impractical. Therefore, fixation was by injection into the lumen of the digestive tract followed by complete immersion of the entire animal in fixative. The digestive tract was freed from its attachments to the abdominal wall and adjacent segments, and fixative was injected into the lumen. After a brief immersion *in situ*, the digestive tract was removed from the abdominal cavity and cut into pieces which were placed into fresh fixative.

Frog heads used for study of the buccal mucosa and glands were decalcified in Von Ebner's fluid.
One general and five analytical histology stains were employed: Harris' hematoxylin-eosin for general observation, Foot's method for reticular fibers, McCallum Verhoff's stain for elastic fibers, Masson's trichrome for collagenous fibers and Gomori's aldehyde-fuchsin for beta cells in the pancreas. Muco-carmin stain was used to show the mucous secretions in the goblet cells.

The stained tissues were dehydrated in an alcohol series and mounted in balsam. Photographs of representative tissue types were made from selected tissue sections.
OBSERVATIONS

Gross Anatomical Features

For the purpose of this study the digestive tract was divided into six anatomical regions: Buccal cavity, esophagus, stomach, small intestine, large intestine and cloaca.

The lips of the buccal cavity are characterized by the rigid skeletal components of the maxilla and premaxilla dorsally and mandible ventrally. These bony structures fit tightly together with the dorsal jaw protruding very slightly beyond the ventral. The buccal cavity is flattened dorsoventrally and thus forms a wide flat portion of the digestive tract. Average measurements made on ten frogs showed that the buccal cavity is more than eleven millimeters wide at the hinge. A connective tissue ridge located midventrally in the floor of the mouth furnishes support and place of attachment for the muscles of respiration and tongue movement. A moat-like groove extending around the anterior and lateral sides of the tongue separates it from the inner surface of the mandible. The tongue is firmly attached to the ventral surface and is capable of some movement, but it can not be protruded.
Two internal nares open into the mouth cavity lateral to the mid-dorsal line. Slightly medial to the internal nares, two rudimentary vomerine teeth form small bony extensions through the oral mucosa. Three millimeters posterior to the vomerine teeth, large convex swellings are formed by the thin walls of the ventral portion of the eye sockets. These eye sockets are lowered during swallowing.

An inclusion of glandular tissue is located posterior to the premaxilla. These glands form a wedge-shaped mass tapering toward the posterior border formed by the internal nares and vomerine teeth (Plate V, fig. 1). The secretions are transmitted to the buccal cavity by numerous ducts ending in the dorsal wall.

The posterior portion of the buccal cavity, known as the pharynx, narrows very sharply into the short esophageal region. A swelling in the mucosal lining on the ventral side of the pharynx locates the glottis. This slit-like opening to the respiratory system is found about two millimeters posterior from the tongue and marks the division between the pharynx and esophagus. The pharynx and esophagus are characterized by numerous longitudinal folds that facilitate the expansion necessary when swallowing.
The stomach is located, for the most part, on the left side of the midventral line and extends from the posterior end of the esophagus to the pyloric sphincter. Displaced by the rest of the viscera, the cardiac flexure lies adjacent to the left abdominal wall. The pyloric portion is turned back toward the midventral line and terminates in the pyloric sphincter. The stomach region, in particular the pyloric stomach, is subject to great size variation according to the degree of distention due to food contents. This expansion is accomplished by the flexibility of the longitudinal folds inherent in the mucosa and submucosa of this region. The average length of the complete stomach, including pyloric and cardiac portions, exceeds sixteen millimeters.

The small intestine, extending from the pyloric stomach to the large intestine, undergoes much coiling along its length. Though this is the longest segment of the digestive tract, its diameter is generally small. An average length, forty-three millimeters, was calculated from measurements made of the small intestine in ten frogs. The small intestine is usually less than two and one-half millimeters in diameter.

The large intestine is readily distinguished from the preceding portion of the digestive tract by its larger
diameter and the absence of flexures or folds. Although
the large intestine characteristically has a diameter
about two times that of the small intestine, its walls
are much thinner. The large intestine is about fifteen
millimeters long and is constricted very abruptly at the
posterior end where it joins the cloaca.

For the purpose of this study three sections of the
cloaca were recognized: an anterior portion, the
coprodeum into which the large intestine empties; an
intermediate part, the urodeum, into which the
urogenital ducts enter; and the terminal proctodeum.
The urodeum receives the ureters which enter through the
dorsal wall. The opening to the urinary bladder is
located on the ventral side. There is a ring of
glandular tissue located at the posterior end of the
urodeum.

The cloaca of the male forms the central component
of the "tail" for which this toad is well known. The
"tail" functions as the copulatory organ and extends
five to eight mm. beyond the posterior surface of the
thighs. The cloaca opens in a large orifice below and
anterior to the tip of this "tail", and the cloaca of
the male *Ascaphus* is approximately four to six mm.
longer than the female cloaca because of the extended
length in the tail.
The common bile duct empties into the small intestine within three millimeters of the pyloric sphincter. It is apparent from the gross dissections that more than one duct joins the pancreas to the intestine. Some of the pancreatic secretions may enter the intestine through the common bile duct that leads from the hepatic and cystic ducts.

The pancreatic tissue is observed as a finely lobulated gland located on the intestinal wall and extending along the length of the bile, cystic and hepatic ducts. The pancreatic tissue is very diffuse and its distribution varies considerably from one specimen to another.

The liver represents the largest organ within the frog. It is grossly divisible into three lobes: the right and the left lateral lobes which flank a smaller medial lobe. Each of these lobes is subdivided into smaller units. The gall bladder is located slightly to the right of the midventral line and is imbedded in a depression of the medial lobe.
MICROSCOPIC ANATOMY

1. Buccal Cavity

A. Buccal Wall

Histologically, the wall of the buccal cavity is divided into two layers: a mucosa of stratified columnar epithelium and an underlying connective tissue component of dense irregular fibers. The epithelium over the skeletal components of the jaw represents the transition zone with the external epithelium of stratified squamous. This transition zone is devoid of glands. The oral mucosa has a ciliated border and numerous goblet cells are interspersed within this layer. The continuity of ciliated surface is interrupted by sensory end-plates of neuro-epithelium (Plate VI, fig. 1). The cells of the plates are characterized by a lack of cilia and by the position of the nuclei. The nuclei are more depressed toward the basal surface of the sensory plaques so that a wide cytoplasmic border exists between the nuclei and the free cell surface.

The submucosa and tunica propria are fused to form one connective tissue layer of irregular collagenous fibers. The tunica propria is very dense and is continuous with the perichondrium in areas such as the
buccal roof, where the mucosa lies over cartilage (Plate V, fig. 1). The submucosa becomes well developed in glandular areas where it forms the stroma between secretory cells.

B. **Tongue**

The sides and lower surface of the tongue are smooth and lined with a stratified ciliated columnar epithelium containing numerous goblet cells, similar to the oral mucosa proper. In contrast, the mucosa on the dorsal surface of the tongue is developed into numerous tall narrow filiform and fungiform papillae (Plate IV, fig. 3). These papillae are lined by a simple columnar epithelium with exception to the flattened tops of the fungiform papillae that are covered with neuro-epithelium. Simple tubular glands extend downward between these papillae. The core of each papilla is formed by numerous fine extensions of tunica propria. Numerous fibroblast cells are dispersed between the collagenous and reticular fibers, forming a typical areolar connective tissue.

The submucosa of the tongue is highly vascularized and shows a predominance of collagenous fibers. Deep within the tissue of the tongue these collagenous fibers are developed into thin walled septa, separating very
large lymph sinuses. These unique structures have numerous bands of intrinsic and extrinsic skeletal muscle embedded within the septa walls. Most of these septa and muscular components traverse the tongue in a dorso-ventral direction.

C. Glands

Two types of alveolar glands are found in the submucosa located anterior to and surrounding the internal nares (Plate V, fig. 1). The anterior portion of this secretory tissue, the intermaxillary gland, is located in the prenasal and subnasal spaces. This rostral extremity located just posterior to the premaxilla bone is about one hundred and fifty micra thick and tapers to a point just anterior to the vomerine teeth. The secretory products from this gland are emptied into the buccal cavity in two areas: approximately four ducts pierce oral mucosa near the anterior border of the internal nares, numerous other ducts enter the buccal cavity near the prenasal cartilage. Some glandular tissue is observed in close association with the prechoanal sac but no indication is found suggesting that part of the secretion empties into the nasal passage. A very light areolar connective tissue is interspersed between the branched alveoli of this
glandular tissue. The nuclei of the secretory cells are depressed toward the basement membrane.

The anterior extremity of the small pharyngeal gland (Rachendruse) is associated with the posterior border of the intermaxillary gland (Plate V, fig. 2). The pharyngeal gland is more diffuse and empties its secretions into the buccal cavity through numerous ducts near the vomers.

These glandular tissues were differentiated as shown by their reaction to the same stains. The following table contrasts the two glands.

<table>
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<tr>
<td>Harris' Hematoxylin</td>
<td>Bright red granules in the cytoplasm</td>
<td>Very homogenous pink cytoplasm</td>
</tr>
<tr>
<td>Masson's trichrome</td>
<td>Bright red cytoplasm</td>
<td>Cytoplasm is light purple</td>
</tr>
<tr>
<td>Muco-carmine</td>
<td>Very faint carmine stain -- indicating a weak positive reaction</td>
<td>No mucus stained</td>
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2. Esophagus

The anterior end of the esophagus is formed by a purse-string constriction of the posterior part of the buccal cavity and pharynx. Thus the esophageal wall
is developed into numerous primary longitudinal folds, involving the mucosa and submucosa.

The esophageal mucosa of stratified columnar epithelium contains many goblet cells. There is an apparent increase in unicellular mucous glands when progressing posteriorly from the mouth and pharynx. A ciliated border covers the epithelium of the esophagus. No sensory plates are found in the pharynx or esophagus.

The epithelium rests on a very dense connective tissue membrane which contains elastic as well as collagenic fibers. This connective tissue sheet is about one half the total wall thickness. Numerous myelinated nerve fibers extend along the esophagus and penetrate the muscularis, following the pathway of the capillary bed in the submucosa. Esophageal glands are not found in *Ascaphus*.

The muscularis externa consists of an inner circular component of smooth muscle and an outer longitudinal portion composed of skeletal muscle fibers. There is an inverse relation between skeletal and smooth muscles. The inner circular layer starts as a very meager band of smooth muscle fibers near the glottis and becomes progressively thicker toward the posterior end of the esophagus where it joins the muscularis externa of the
stomach. The skeletal muscle component gives way to smooth muscle near the anterior end of the stomach. The muscularis accounts for one fourth to one sixth of the total thickness of the esophageal wall.

The somatic muscles are tightly bound to the muscularis externa of the esophagus by an adventitia. The coarse collagenous fibers composing this layer are also interlaced with blood vessels and nerves. This connective tissue becomes continuous with the epimysium of the surrounding neck muscles.

3. Stomach

The stomach wall is composed of four very distinct and well developed integral layers: the mucosa, submucosa, muscularis and serosa. The first two of these layers enter into the formation of longitudinal folds or rugae characteristic of the stomach. The epithelial component of the mucosa is of two types, secretory and protective (Plate I, fig. 1). Simple columnar epithelium with a very thick (about fifteen micra) cuticular layer forms the gastric mucosa. The mucosa is interrupted by numerous gastric pits into which the tubular gastric glands open. An area containing a high concentration of very large unicellular mucous glands is found at the neck of the
tubular glands, separating the epithelium of the gastric pits from that of the tubular glands (Plate I, fig. 2). These glands are lined with a secretory type of simple columnar epithelium. The glandular cells have a large, round, centrally located nucleus and a granular cytoplasm. Eighty per cent of these glandular cells have small dense staining nuclei of wandering lymphocytes in association with the basal membrane (Plate I, fig. 3).

A tunica propria of areolar connective tissue underlies these epithelial types. This connective tissue forms the support for the gastric mucosa and extends between the tubular glands; it serves as the pathway through which the many capillaries travel. Two cell types are differentiated in this connective tissue: the histiocyte with a compact dense staining nucleus and the fibroblast with a very large diffuse nucleus. Both reticular and collagenous fibers are present in this connective tissue, the reticular fibers being the most numerous.

The muscularis mucosa, a smooth muscle component of the mucosa, is divided into two parts: the inner circular and outer longitudinal muscle bands. The outer longitudinal muscle band is slightly thinner than the inner circular band. The complete muscularis mucosa is about thirty micra thick.
A very thick submucosa of areolar connective tissue separates the muscularis mucosa and the muscularis externa. Longitudinal folds or rugae of the stomach wall are supported by this connective tissue. Thus these folds involve all of the components of the mucosa over a core of submucosa. The rugae become flattened when the stomach is distended by food contents. The necessary elasticity for this distension is supplied by the numerous elastic fibers in the submucosa. Reticular and collagenous fibers are also present. Fibroblasts and histiocytes are interspersed between the fibrous components of the submucosa.

A circular layer of smooth muscle forms a very thick muscularis externa. The outer longitudinal component is almost nonexistent. The complete muscle mass is between two hundred fifty and three hundred fifty micra thick depending upon the state of contraction.

The serosa is composed of simple squamous mesothelium and the associated thin layer of connective tissue that binds it to the outer surface of the muscularis externa. Numerous capillaries and non-myelinated nerves are located in the connective tissue between the smooth muscle and the peritoneum.
4. **Small Intestine**

The pyloric opening is guarded by a flap of tissue formed by an inward folding of the intestinal wall. This fold of tissue is composed of the mucosa and submucosa. The anterior end of this flap is free and extends back into the stomach about two and one-half millimeters. Thus it serves as a valve and allows the stomach contents to enter the small intestine without any back-flow (of the intestinal contents) into the stomach. The muscularis externa is augmented by additional muscle fibers in this region of the pyloric valve. A distinct muscle mass may not be distinguished as the pyloric sphincter, but rather the circular muscle band of the muscularis externa is broadened by additional muscle fibers.

The wall of the small intestine shows the usual division into mucosa, submucosa, muscularis externa and serosa. Its interior wall is thrown into longitudinal primary folds, involving the mucosa and submucosa. The mucosal epithelium is a simple columnar epithelium interspersed with numerous goblet cells. This epithelial component is about fifteen micra thick. Lymphocytes are plentiful in this epithelial lining; however, there is no indication that they become aggregated or grouped in nodules. They are dispersed evenly throughout the epithelial layer.
Very large cells with centrally located nuclei represent a very unique epithelial inclusion. These cells are located at the crest of intestinal folds and are about four times larger than goblet cells (Plate II, fig. 1). Their cytoplasm is devoid of a strongly acid mucus as shown by its failure to stain with mucocarmine.

The mucosa of the small intestine is supported by longitudinal folds in the underlying connective tissue. The tunica propria and submucosa are fused into one connective tissue sheet due to the absence of any muscularis mucosae. Many smooth muscle fibers are dispersed within this dense irregular connective tissue. These muscle fibers are oriented perpendicular to the lumen in such a way as to suggest that they function in withdrawing the folds. In addition to the usual inclusion of capillaries, arteries and veins, this connective tissue affords passage for the lymphatic system including large chyle sinuses that extend into the folds.

The muscularis externa, as usual, is composed of an inner circular layer and an outer longitudinal layer. The circular muscle band accounts for the total thickness (about eighty micra) with exception of a layer three cells thick that represents the longitudinal muscles. An
occasional node of lymphatic tissue encroaches upon the muscle cells of the muscularis externa. Such diffuse lymph patches are not prevalent in Ascaphus. Non-myelinated nerves of Auerbach’s plexus, are observed in association with the muscularis externa and are located between the inner circular and outer longitudinal layers of smooth muscle.

The serosa is composed of a very thin connective tissue sheet underlying a simple squamous mesothelium. This simple squamous epithelium is continuous with the lining of the peritoneal cavity.

5. Large Intestine

The transition of small intestine to large intestine is very abrupt. No sphincter or valve marks this junction. Microscopically, the wall of the large intestine shows four major divisions: mucosa, submucosa, muscularis and serosa. The large intestine has no folds and the submucosa and muscularis externa are much reduced.

The total thickness of the colonic intestine wall is about one hundred fifty micra thick and about half (seventy micra) of this thickness is the mucosa (Plate III, fig. 2). The epithelium lining the lumen is a very high
simple columnar epithelium containing great numbers of goblet cells. The surface of this epithelial lining is marked by a cross hatching of grooves and shallow fissures. The nuclei are depressed toward the basal surface of the epithelium leaving a wide band of cytoplasm and mucin near the free cell surface. The nuclear arrangement gives the impression of being very complex due to the many lymphocytes present in this area. These lymphocytes can be distinguished by round nuclei that stain somewhat more basophilic than the nuclei of the secretory cells.

The submucosa is made up of irregularly disposed connective tissue including elastic and coarse collagenous fibers. A rich supply of blood and lymph vessels traverse this zone. The submucosal thickness is about one fourth to one half that of the entire large intestine.

A muscularis externa, composed of inner circular and outer longitudinal muscle fibers, constitutes a very thin layer of smooth muscle. The depth of muscularis comprises nearly one-fourth of the large intestine wall and about thirty micra in thickness.

A serosa of simple squamous epithelium overlies a very thin layer of coarse collagenous fibers. This layer comprises less than one tenth of the total thickness of the wall.
6. Cloaca

The cloaca is divided into three histological components: mucosa, submucosa, and muscle. The mucosa lining the anterior divisions of the cloaca (coprodeum and urodeum) possesses a simple ciliated columnar epithelium, containing many goblet cells (Plate III, fig. 1). A ring of alveolar glands (more highly developed in the male) encircle the lumen between the urodeal and proctodeal portions. Within this ring of glandular tissue the simple columnar epithelium of the urodeum comes in abrupt contact with the stratified squamous epithelium of the proctodeum. By virtue of its position at the terminal end of the digestive tract, the proctodeal portion of the cloaca represents a transition from digestive mucosa to external epithelium. The epithelium lining the anterior proctodeum in the male frog is developed into cornified spines.

The submucosa is a loose irregular connective tissue composed of coarse collagenous fibers between numerous blood vessels and myelinated nerves. In the male frog this layer becomes greatly modified as cavernous tissue posterior to the pelvic symphysis. This cavernous tissue is analogous but not homologous to the corpora cavernosa penis of mammals.
Two muscular components are readily identified; a thin circular band of smooth muscle and the compressores cloacae. The circular layer is composed of a band about ten micra thick formed by intermittent fibers of smooth muscle. Cavernous tissue surrounds this muscle layer in the male frog. The gliding planes of Van Dijk, extensive U-shaped sinuses, are located on the dorsal and ventral outer surfaces of this smooth muscle band and separate it from the compressores cloacae dorsally. The compressores cloacae are paired muscles that join mid-dorsally forming an arch through which the posterior half of the cloaca passes. The ends of these striated muscles attach to the rudis nobleiana, a scissors shaped bone on the ventral surface of the cloaca. Contraction of the muscles compress the cloaca against the rudis nobleiana.

7. Liver and Gall Bladder

The liver is organized into a system of anastomosing hepatic tubules, supported by a very light stroma of reticular connective tissue and enclosed in a connective tissue capsule. The lack of lobular development gives this gland a very homogeneous appearance, interrupted only by numerous large pigment cells located in the stroma. A thin layer (about three cells thick) of hemopoietic tissue
underlies the connective tissue capsule. Additional hemopoietic tissue may be found aggregated into small patches around the main branches of the hepatic ducts (Plate II, fig. 2 and Plate VI, fig. 2).

The hepatic ducts are identified by a lining of simple columnar epithelium of varying height. These ducts frequently have a ciliated border. Components of the circulatory system accompany these ducts in their tortuous course through the gland.

On the cellular level, the hepatic cells are radially distributed around the branching tubules. Six to eight of these very large pyramidal cells (seven cells are most frequent) encircle each hepatic tubule (Plate IV, fig. 2). The nuclei are most frequently displaced toward the lumen and numerous very large lipid vacuoles exist within the cytoplasm. Secretory granules are unevenly clumped within the hepatic cells making the observer cognizant of a secretory function. The phagocytic cells of von Kupffer are observed within the hepatic sinusoids (Plate IV, fig. 2).

The wall of the gall bladder is lined by a simple cuboidal epithelium. This epithelial lining rests on a very thin layer of connective tissue; interspersed within this connective tissue numerous smooth muscle fibers may
be seen. The complete wall of the gall bladder is about twenty micra thick.

8. Pancreas

The pancreatic tissue is divided into many loosely packed lobules that are separated by highly vascularized connective tissue. Approximately one cubic millimeter of pancreatic tissue is characteristically found within the capsule of the liver. This inclusion of tissue is located near the base of the gall bladder and around the hepatic duct (Plate VI, fig. 2). No connective tissue septum separates the liver from the pancreatic tissue. The structure and staining reactions are similar to the observations made on the other pancreatic tissue.

Islets of Langerhans are found interspersed as distinct lobules within the connective tissue septa of the mesenteric pancreas. None were observed within the pancreatic tissue contained by the liver capsule.
DISCUSSION

Some consideration must be given to the adaptive modifications of the alimentary tract as they relate to the nature of the diet and feeding habits. Van Denburgh thought that beetles and spiders comprise the greatest bulk of the Ascaphus diet. Direct observations must not have preceded this assumption by Van Denburgh. He wrote, "These organisms may be hunted under water and hence probably in pools where the water does not flow very swiftly and hydrostatic organs could function well and be of use in hunting the prey" (20, p. 262). After a similar investigation DeVilliers concluded, "If the usually accepted theory of the function of the buccal glands is correct, Ascaphus must be an active insect hunter" (6, p. 7). These indirect methods of theorizing about the diet are not conclusive but they are attempts to assign function to the structures found. The stomach contents examined in conjunction with this study were in a state of advanced disintegration and nothing could be identified.

The motility and morphology of the frog tongue appears to be associated with the habitat in which it lives. In most Salientia the tongue is attached to the front angle of the jaw and capable of projection. However, tongue
movement of thoroughly aquatic forms is greatly reduced (13, p. 201). Ascaphus, a form closely associated with an aquatic environment, falls in this latter class showing very limited tongue motility. The fact that the tongue of Ascaphus is not protrusible must have some relevance to the feeding habits. If the tongue motility is an adaptive mechanism for catching flying insects, as some have suggested, then it may be concluded that the diet of Ascaphus does not commonly include these insect forms. The large septate lymph sinuses found in the tongue of this frog apparently have not been described for other species. The sinuses could provide a method for making the tongue rigid while the skeletal muscle fibers interspersed in the septa could allow for movement of the tongue. However, no attempt was made to verify this theory or to suggest any function.

Noble (13, p. 202) reports that cilia are present on the tongue and palate in terrestrial Amphibia but are lacking in larvae and in some aquatic Urodeles. The ciliated epithelium was well developed on the palate but no cilia were found on the tongue of Ascaphus. Cilia are absent from the surface of the numerous end-plates of neuro epithelium located on the buccal wall.
Oeder (14) first showed the dual nature of the buccal glands while working on Bufo, although Gaupp had earlier suspected their double origin. Cohn (4, p. 719-734) studied the comparative morphology of these glands using nine genera of frogs. DeVillliers described the cranial anatomy of Ascaphus and wrote, "There is nothing very unusual in the organs encountered in the nasal region, and particularly nothing that can be considered as strikingly primitive" (6, p. 7). A closely related New Zealand frog was the subject of a similar publication by Stephenson (18, p. 255-305). She found that the structure of the buccal glands was modified only slightly from that found in Ascaphus. From the numerous histological studies made on the buccal glands and their origin, DeVillliers concluded that the pharyngeal gland (Rachendruse) was originally scattered over a large area of the pharynx and that it is in the process of reduction (6, p. 15). The very small pharyngeal gland located just posterior to the intermaxillary gland in the material studied could be used in support of this hypothesis.

Cohn (4, p. 730) recognized the dual origin of the intermaxillary and pharyngeal glands but he thought they had a similar secretory function. On the basis of the
staining reactions observed in this study, it would appear that the secretory products obtained from these glands were of different chemical composition, and therefore, perform a different function. The production of an adhesive substance for the tongue is most frequently attributed to the intermaxillary glands. DeVilliers (6, p. 9) described the presence of large quantities of this adhesive secretion in the prechoanal sac. Failure of these glands to stain with muco-carmine offers strong evidence that if the glandular products are mucoid, strong acid groups such as sulfates or hyaluronic acid are not present. Some neutral and basic mucoids are able to change the color of susceptible dyes; this metachromasia is apparently caused by the polymerization of the dye molecules, which produces a change in color (5, p. 38-39). The carmine stain used in this study is one of the susceptible dyes, therefore, this dye can not be used to demonstrate the presence of metachromatic mucoids.

Esophageal glands are found in Rana and Bufo but do not occur in various Salientia or in certain Urodeles (13, p. 202). These glands are not found in Ascaphus or closely related genera. An attempt was made to correlate the relationship of the genera having esophageal glands,
however, the complexity of the relationships indicate that this problem is deserving of more extensive investigation.

The gastric structures such as the cuticular border, large goblet cells and tubular glands found in _Ascaphus_ are similar to the generalized description of frog histology given by Krause (9, p. 549-552). The gastric glands of frogs are distinctively less zymogenic than those found in other Amphibia (2, p. 102).

Lymphocytes are found in association with the base of the secretory cells lining the simple tubular gastric glands and appear to be located intracellularly between the epithelial nuclei and the basement membrane. Kelsall (8, p. 402) observed the lymphocytes in the intestinal mucosa of hamsters and found that more than ninety-six per cent were within the epithelial cells adjacent to the basement membrane. The presence of intracellular lymphocytes in the intestine of other animal types has also been shown (19, p. 262 and 1, p. 81).

Noble (13, p. 204) reported that the small intestine of most frogs is sharply separated from the stomach by a pyloric constriction. The lack of a well developed sphincter in _Ascaphus_ suggests that some other mechanism may be used to retard the movement of food.
The pyloric opening of the stomach is partially filled with an anteriorly directed fold of gastric mucosa which may function as a valve in stomach emptying. How this fold could eliminate the need for a well developed ring of circular muscle is open to question.

An unknown cell type found in the intestinal mucosa may be readily distinguished from adjacent goblet cells by the structure and staining reaction. Two or more small dense nuclei are located in the center of these large cells. They do not stain with muco-carmine and stain very lightly with other techniques suggesting that the solvents used in fixation and staining may dissolve the vacuolar contents from the cell.

Pyloric type glands are found between the folds located in the small intestine of urodèles but are not present in the intestine of Salientia (13, p. 204). There were no glands of this type found in Ascaphus.

Structurally, the large intestine of Ascaphus is very similar to the large intestine of other primitive Amphibians. There is no distinct fold or valve separating the small intestine and large intestine of Ascaphus. In some Salientia this transition zone is developed into a valve or ring fold (13, p. 204). Two divisions have been recognized in the large intestine of some species of
frogs: an anterior large reservoir for feces and a posterior more muscular part (13, p. 204). The large intestine of *Ascaphus* can not be subdivided in this manner.

A very comprehensive discussion of the cloaca, its development and the associated skeletal components was given by Van Dijk (21 and 22). He constructed wax models of the pelvis and gave a detailed account of the morphology. The discussion on the cloaca shall, therefore, be limited to deviations or additions that can be made to the observations of Van Dijk.

No reference was found to suggest that Van Dijk saw ciliated epithelium lining the anterior portion of the cloaca. The presence of cilia on the cloacal border such as found in *Ascaphus* is not common among frogs and it is interesting to speculate on the possible function. The fact that *Ascaphus* is one of the few frogs having internal fertilization might lead one to postulate that the cilia function in the reproductive process. However, it would also be necessary to speculate on the functions of the secretory products of the numerous goblet cells and the cloacal glands found in this region. The intense positive reaction for acid mucoids produced in the goblet cells indicates that ciliary movement would become ineffective in moving the cloacal contents should they be
covered by that mucus. On the other hand the negative reaction of the contents of the cloacal glands leads one to presuppose a more fluid non-mucous secretion which could facilitate the movement of cloacal contents in fertilization.

The histogenesis of liver and pancreatic tissue is very similar. Noble summarized the formation of these glands as follows: "The pancreas arises near the liver in the form of three outgrowths from the intestinal wall, which soon fuse to form a single structure" (13, p. 205). The distal portions of the outgrowths form glands of the tubular type; the glandular tissue maintains its attachment to the intestinal wall by ducts that empty into the intestine.

Two pancreatic ducts empty into the intestine of adult Urodeles, the anterior behind the pylorus and a posterior in association with the hepatic duct (13, p. 205). The Salientia, according to Noble, lose the anterior duct during embryonic development. Only one pancreatic duct was found in the specimens observed in this study. The posterior duct may be the only pancreatic duct found in Ascaphus, however, histogenic studies on the larval stages would be required before such a conclusion could be made.

The incorporation of pancreatic tissue within the capsule of the liver could be due to the incomplete
separation of these glands from their common embryonic origin, however, once again histogenic studies should be made to verify this theory.

The liver of Urodeles differs from that of frogs by the presence of hemopoietic tissue under the connective tissue capsule. The liver of *Ascaphus* is well supplied with hemopoietic tissue, a characteristic that may also accentuate its phylogenetic relationship.
CONCLUSIONS

The data presented here indicate that *Ascaphus truei* may be classified as a neotenic form of the Salientia. It would appear that *Ascaphus* has retained some morphological remnants (i.e. hemopoietic liver, reduced pyloric sphincter and reduced tongue motility) of the ancestral amphibian while at the same time becoming highly specialized for its aquatic environment. Support for this conclusion may be found in previously published anatomical studies on the skeletal and nervous systems.
SUMMARY

A study of the macroscopic and microscopic anatomy of the digestive tract and associated glands of *Ascaphus truei* was presented. The digestive tract was divided into six anatomical regions: buccal cavity, esophagus, stomach, small intestine, large intestine, and cloaca.

1. The dorsal and lateral surfaces of the buccal cavity are lined with stratified ciliated columnar epithelium interspersed with many sensory end plates.

2. The tongue is capable of limited movement, and it cannot be projected anteriorly beyond the lips. Very large lymph sinuses are found deep within the tongue which may function as erectile tissue.

3. The intermaxillary and pharyngeal glands occur in the buccal cavity. They are best distinguished by staining reactions; in neither case are the secretions strongly positive for acid mucoids.

4. The stomach wall is lined with a simple columnar epithelium capped with a very thick cuticular border.

5. Simple tubular gastric glands extend from the base of the mucosal folds. Large mucous cells separate these tubular glands from the cuticular epithelium. Many lymphocytes are associated with the base of the columnar cells lining the tubular glands.
6. Very large cells found scattered throughout the mucosa of the small intestine have centrally located nuclei and apparently do not secrete mucus. The function of these cells is not known.

7. The mucosal epithelium of the large intestine is a very tall simple columnar type containing numerous goblet cells.

8. The first two divisions of the cloaca, coprodeum and urodeum, are lined with a simple ciliated columnar epithelium.

9. Proctodeal glands are found between the urodeum and proctodeum. A muco-carmine stain failed to reveal any mucous secretion.

10. Hemopoietic tissue is found in the stroma underlying the liver capsule.

11. Some pancreatic tissue is located within the liver capsule, near the base of the gall bladder.

When one compares the digestive tract of *Ascaphus truei* with those of Salientia and Urodeles he may conclude that this frog is a highly modified form, showing many characteristics of the ancestral amphibian from which the frogs and salamanders are believed to have evolved.
BIBLIOGRAPHY


Figure 1. Cuticular border (cu) of the protective epithelium of the gastric mucosa. Tubular glands extend from the base of the epithelial folds.

Figure 2. A high power photomicrograph of the gastric mucosa showing the relationship between protective epithelium and tubular glands (gl). Large mucous glands (mu) separate the secretory epithelium of the tubular glands from the protective epithelium.

Figure 3. Two types of nuclei present in the secretory cells of the gastric glands. The small dark nuclei near the basal membrane mark the location of lymphocytes within the secretory epithelium. This photomicrograph was made from a slide stained by Foot's technique.

Figure 1. One of the very large cells (arrow) found in the epithelium of the mucosa of the small intestine. Note that the cell is much larger than the two adjacent goblet cells. Two intercellular lymphocytes may be recognized by their cytoplasmic halos on the left side of the picture.

Figure 2. Hemopoietic tissue observed around the walls of all major hepatic ducts. The duct is lined with a simple columnar epithelium.

Figure 1. The simple ciliated columnar epithelium of the cloaca (cl). The numerous goblet cells may be seen as light areas within the epithelial lining. Many nerves and blood vessels are found in the submucosa.
Figure 2. The very tall simple columnar epithelium lining of the large intestine. Many goblet cells are interspersed within this epithelium.

PLATE IV

Figure 1. High power photomicrograph (1200X) of a single proctodeal gland. This print was photographically manipulated to increase contrast of surrounding connective tissue.

Figure 2. A typical liver lobule composed of seven cells. Note the cell of Von Kupffer found within the lobule. Many vacules may be seen within the hepatic cells. (1200X)

Figure 3. Two types of papillae found on the tongue surface. The fungiform papillae are topped with neural epithelium and present a broad, club-shaped end. The filiform papillae are tall and slender. (1200X)

PLATE V

Figure 1. Buccal glands of two types may be distinguished in this longitudinal section of the head. The staining reaction of the intermaxillary gland is typical of hematoxylin and eosin proper; the posterior pharyngeal glands are set off by a greater cytoplasmic eosinophilia. (55X)

Figure 2. An enlarged photomicrograph (550X) showing the staining reaction of the buccal glands when Masson's trichrome is used. The intermaxillary gland is on the left; pharyngeal gland is on the right.

PLATE VI

Figure 1. Epithelium of the buccal wall. The stratified ciliated columnar epithelium is interrupted by sensory end-plates of neuro-epithelium. (1200X)
Figure 2. Intrahepatic pancreas; pancreatic tissue found within the liver capsule. Note the hemopoietic tissue around the cystic duct. Dark pigment cells may be seen in the hepatic tissue.
Figure 1

Figure 2
PLATE VI

Figure 1

Figure 2